

BOVINE HOMOLOGUE OF  $\beta_2$ -MICROGLOBULIN ISOLATED FROM MILK

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Summary: Lactollin is a bovine milk protein previously isolated, characterized, and crystallized. Comparison of its minimum molecular weight (12,000), amino acid composition, and sequence of the first 32 residues and those of  $\beta_2$ -microglobulins isolated from several species, indicates that lactollin is bovine  $\beta_2$ -microglobulin.

Human  $\beta_2$ -microglobulin is a small protein with a molecular weight of 11,800. It is present in different body fluids and on the surfaces of various cell types (1, 2). The amino acid sequence of this protein has been determined and the homology to the constant region of IgG light and heavy chain domains has been demonstrated (3).  $\beta_2$ -Microglobulin is also identical to a small peptide that is bound to HL-A histocompatibility antigen (4). The homologues of  $\beta_2$ -microglobulin in man (3, 5), rabbit (6, 7), dog (8), guinea pig (9), and mouse (10, 11) have been isolated and their composition and/or partial amino acid sequences have been compared.

Recently Cejka and Kithier (12) isolated  $\beta_2$ -microglobulin from human milk and determined that its concentration in colostrum and milk is highest at the beginning of lactation. We previously isolated lactollin, a crystalline protein, from bovine milk; it also appears to be present in colostrum at a significantly higher concentration than in normal milk (13, 14, 15). Although a molecular weight of 43,000 was calculated from the sedimentation pattern of lactollin, its minimum molecular weight determined from the amino acid composition was 11,000 (13). This value, together with a comparison of the amino acid composition of lactollin with that of the  $\beta_2$ -microglobulin isolated from other species, indicates a high degree of homology. In the present communication, we report the partial amino acid

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sequence of bovine lactollin and show it to be similar to  $\beta_2$ -microglobulin homologues in other species.

Materials and Methods: Isolation of lactollin from bovine milk and colostrum was as described (13, 14) except that the DEAE-cellulose fractionation was carried out at 3°C. Also, gel filtration was used to isolate lactollin from the crude lactollin fraction. In one experiment, 2.0 g of the red fraction obtained from colostrum and precipitated by ammonium sulfate (13), upon chromatography on DEAE-cellulose yielded 42% of the protein with the starting buffer of 0.005 M sodium phosphate, pH 8.3. About 80% of this fraction was lactoferrin eluted with the front while lactollin and some lactoferrin accounted for most of the other 20% found in the trailing portion of this peak. This fraction was dissolved in 0.025 M sodium acetate, pH 5.0 and subjected to gel filtration on a Biogel P60 column, 2 by 61 cm, 3°C, equilibrated with the same buffer. The final purification of lactollin is accomplished by crystallization (13).

Lactollin was reduced and alkylated by a modification of the method of Shechter et al. (16). During the course of the reaction  $N_2$  was bubbled through the solution. At the end of the reaction the S-carboxymethylated (SCM) lactollin was separated from the reagents by dialysis at 3°C in the dark.

Disc gel electrophoretic analysis of lactollin and SCM-lactollin was by standard gels, pH 9.6, 4 M urea, and at pH 4.3, 8 M urea (17). The molecular weight of lactollin was estimated by electrophoresis with 10% gels containing sodium dodecyl sulfate, both in the presence and absence of mercaptoethanol (18). The amino acid composition is from an earlier publication (13) except that the values are based on molar ratios, taking phenylalanine as four.

Amino acid sequence determination was on a Beckman 890C sequencer with a Quadrol double cleavage program. Identification of the phenylthiohydantoin (PTH) amino acids was accomplished by gas and thin-layer chromatography (19, 20) and/or amino acid analysis after back hydrolysis with HI to the parent amino acid after Smithies et al. (21).

Results and Discussion: Gel filtration on Biogel P60 of the fraction rich in lactollin eluted from DEAE-cellulose is shown in Figure 1. Lactoferrin was eluted in the first peak followed by lactollin, peak 2. The protein in peak 3 has not been further characterized. Yield of lactollin was 39 mg. A small sample of lactollin, 0.1 mg dissolved in 0.025 M sodium phosphate pH 8.2, on standing at 3°C gave typical crystals of lactollin (13).

Disc gel electrophoretic patterns of lactollin and SCM-lactollin are shown in Figure 2. SCM-lactollin shows a single band at pH 4.3; at pH 9.6 it shows two zones and in some gel patterns three. Berggård and Bearn (1) found similar results for human  $\beta_2$ -microglobulin by starch gel electro-

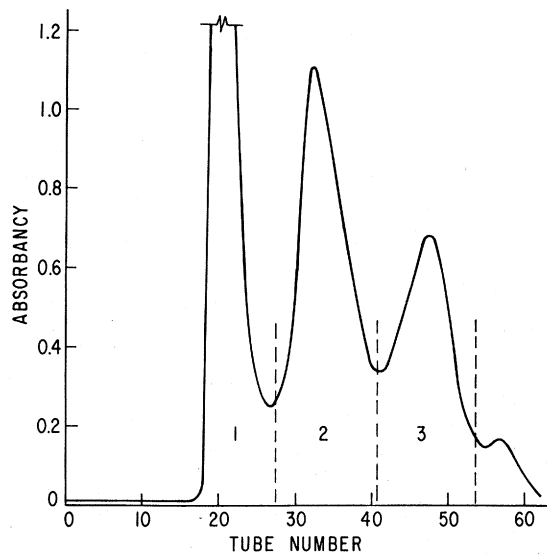


Figure 1. Gel filtration of the fraction from DEAE-cellulose rich in lactollin on Biogel P60, 2 by 61 cm at 3°C. The protein, 150 mg, was dissolved in 10 ml 0.025 M sodium acetate pH 5.0 and layered on the column. Fractions of 4 ml were collected at a rate of 12 ml/hour. Dashed lines indicate the fractions combined.

phoresis, 8 M urea at acid pH, in which the native and reduced, alkylated  $\beta_2$ -microglobulin each showed one zone. The reduced protein had the slower mobility. Starch gel electrophoresis in 8 M urea at alkaline pH also showed one zone for  $\beta_2$ -microglobulin while the reduced, alkylated protein gave two zones. The number of zones increased to three when either sample stood in the urea buffer, 3°C, for a few days before electrophoresis.

Molecular weights were determined for lactollin, reduced lactollin, and SCM-lactollin by sodium dodecyl sulfate gel electrophoresis. Six determinations showed an average molecular weight of 13,400 (varying from 12,400 to 13,700) with no significant difference for the native and modified protein. This approximates the 12,000 molecular weight based on amino acid data (Table I) and the values reported for human  $\beta_2$ -microglobulin (1).

The amino acid composition of  $\beta_2$ -microglobulins from various species

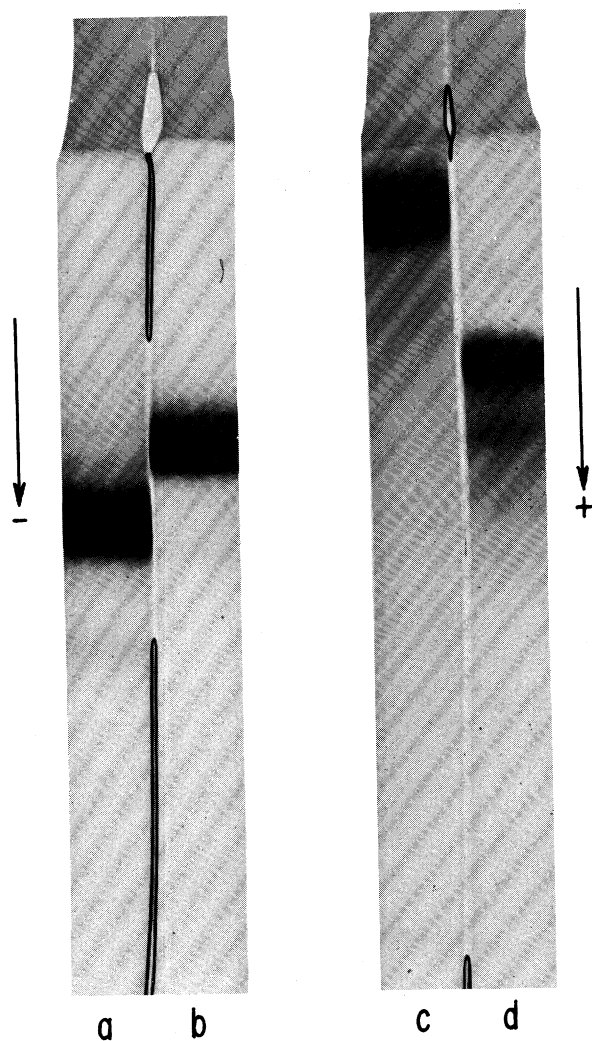


Figure 2. Disc gel electrophoretic patterns of lactollin a,c and SCM-lactollin b,d. Gels at pH 4.3 and 8 M urea are a and b and gels at pH 9.6, 4 M urea are c and d.

are compared with that of lactollin in Table I. The half cystine and tryptophan residues are invariant while lysine, histidine, glutamic acid, phenylalanine, and glycine vary by no more than one residue in all species.

Comparison of the N-terminal sequence of mouse, human, dog, and rabbit  $\beta_2$ -microglobulin with cow lactollin provides further evidence that lactollin is  $\beta_2$ -microglobulin (Fig. 3). The close relationship of this protein from

TABLE I  
AMINO ACID COMPOSITION OF  $\beta_2$ -MICROGLOBULIN FROM VARIOUS SPECIES  
AND OF COW LACTOLLIN

Amino acid	$\beta_2$ -Microglobulin				Lactollin
	Human (1)	Guinea pig (9)	Rabbit (6)	Mouse (10)	Cow <sup>a</sup> (13)
Aspartic acid	12	14	15	10	11
Threonine	5	3	4	7	2
Serine	10	9	6	7	8
Glutamic acid	11	10	11	11	12
Proline	5	7	8	8	9
Glycine	3	4	3	4	3
Alanine	2	4	2	5	1
Half-cystine	2	2	2	2	2
Valine	7	9	10	5	5
Methionine	1	1	1	4	0
Isoleucine	5	5	3	6	6
Leucine	7	7	7	4	8
Tyrosine	6	4	5	4	6
Phenylalanine	5	5	5	4	4
Lysine	8	8	8	9	9
Histidine	4	5	4	4	4
Arginine	5	3	4	4	5
Tryptophan	2	2 <sup>b</sup>	2	2	2

<sup>a</sup>Values represent published data (13) based on molar ratios, taking phenylalanine as 4.

<sup>b</sup>Assumed value.

the five species is reinforced by the fact that 22 of the first 32 amino acid residues are invariant.

Lactollin contains four subunits based on a sedimentation molecular

	<u>1</u>									<u>10</u>
Cow	Ile	GLN	Arg	Pro	PRO	Lys	Ile	GLN	VAL	TYR
Mouse	Ile	GLN	Lys	Thr	PRO	Gln	Ile	GLN	VAL	TYR
Human	Ile	GLN	Arg	Thr	PRO	Lys	Ile	GLN	VAL	TYR
Dog	Val	GLN	His	Pro	PRO	Lys	Ile	GLN	VAL	TYR
Rabbit	Val	GLN	Arg	Ala	PRO	Asn	Val	GLN	VAL	TYR
	<u>11</u>									<u>20</u>
Cow	?	ARG	HIS	PRO	Pro	GLU	?	GLY	LYS	Pro
Mouse	SER	ARG	HIS	PRO	Pro	GLU	ASN	GLY	LYS	Pro
Human	SER	ARG	HIS	PRO	Ala	GLU	ASN	GLY	LYS	Ser
Dog	SER	ARG	HIS	PRO	Ala	GLX	ASX	GLY	LYS	Pro
Rabbit	SER	ARG	HIS	PRO	Ala	GLU	ASN	GLY	LYS	Asp
	<u>21</u>									<u>30</u>
Cow	ASN	Tyr	LEU	ASN	CYS	TYR	VAL	?	Gly?	PHE
Mouse	ASN	Ile	LEU	ASN	CYS	TYR	VAL	Thr	Glu	PHE
Human	ASN	Phe	LEU	ASN	CYS	TYR	VAL	Ser	Gly	PHE
Dog	ASX	Phe	LEU	ASX	CYS	TYR	VAL	Ser	Gly	PHE
Rabbit	ASN	Phe	LEU	ASN	CYS	TYR	VAL	Ser	Gly	PHE
	<u>31</u>									
Cow	HIS	PRO								
Mouse	HIS	PRO								
Human	HIS	PRO								
Dog	HIS	PRO								
Rabbit	HIS	PRO								

Figure 3. Comparison of the N-terminal sequence of cow lactollin with mouse (11), human (5), dog (8), and rabbit (7)  $\beta_2$ -microglobulin. Capital letters represent invariant residues.

weight of 43,000 and minimum molecular weight of 11,000 (13) or 12,000 based on its amino acid composition reported in Figure 1. The subunits are not covalently linked since molecular weights by gel electrophoresis in sodium dodecyl sulfate for both the native and reduced protein agree with the monomeric values. Apparently human  $\beta_2$ -microglobulin is monomeric in solution with a molecular weight of 12,000, based on sedimentation (1). The sedimentation coefficient of  $\beta_2$ -microglobulin is 1.65S compared to 3.25S for lactollin (13). Lactollin, like  $\beta_2$ -microglobulin, contains one cystine residue per monomer since no free sulphydryl groups were detected.

Rossi et al. (22) crystallized fragments resulting from pepsin digestion of IgG protein which were designated Fab'. The Fab' and Fab fragments consist of four domains of the IgG protein which include the light chain with its variable ( $V_L$ ) and constant ( $C_L$ ) domain and the variable ( $V_H$ ) and first constant ( $C_H^1$ ) domain of the heavy chain (23). The balance of the IgG molecule, the Fc fragment, also represents four domains which include the constant region of the heavy chains ( $C_H^3$ ) and ( $C_H^4$ ) and has been crystallized (24, 25). Even though  $\beta_2$ -microglobulin may have evolved independently from the primitive immunoglobulin before gene duplication produced the light and heavy chains of the present IgG molecule, it still retains similar features of the individual domains of IgG protein, including the large amino acid residue loop formed by the cystine residue in the constant domains of the light and heavy chains (3). Since lactollin appears to be bovine  $\beta_2$ -microglobulin, it is conceivable that the tetramer of lactollin associates in a manner similar to that of the Fab' and Fc fragments to produce the crystalline bovine  $\beta_2$ -microglobulin.

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